L8 ANSWER 50 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:405096 BIOSIS
DOCUMENT NUMBER: PREV199598419396
TITLE: Vascular alpha-adrenoceptors: From the gene to the human.
AUTHOR(S): Bylund, David B.; Regan, John W.; Faber, James E.; Hieble, J. Paul; Triggle, Christopher R.; Ruffolo, Robert R., Jr. (1)
CORPORATE SOURCE: (1) Pharmacological Sciences, UW2523, SmithKline Beecham Pharmaceuticals, 708 Swedeland Road, PO Box 1539, King Prussia, PA 19406-0939 USA
SOURCE: Canadian Journal of Physiology and Pharmacology, (1995) Vol. 73, No. 5, pp. 533-543. ISSN: 0008-4212.
DOCUMENT TYPE: General Review
LANGUAGE: English
SUMMARY LANGUAGE: English; French
AB Adrenoceptors can be subdivided into three major types, the alpha-1-, alpha-2-, and beta-adrenoceptors. Each of these types can be further subdivided into three subtypes, based on pharmacological characteristics. Molecular cloning techniques have supported this subclassification. Recent data now suggest that alpha-adrenoceptor subtypes identified by pharmacological and molecular techniques correspond well, although species orthologs of several adrenoceptor subtypes have been identified. The secondary structure of the adrenoceptors has been elucidated and correlated with their interaction with second messenger molecules. alpha-1-Adrenoceptors, beta-adrenoceptors, and alpha-2-adrenoceptors mediate their actions through stimulation of inositol phosphate release, stimulation of adenylate cyclase, and inhibition of adenylate cyclase, respectively. Site-directed mutagenesis and the preparation of **chimeric** receptors have located the site of receptor - second messenger interaction to the third **intracellular loop** for each of these adrenoceptors. While subtypes of each of these classes all interact with the same second messenger, studies with recombinant alpha-2-adrenoceptors show subtype-related differences in receptor second messenger interaction. Multiple alpha-adrenoceptor subtypes are expressed in vascular smooth muscle and are involved in various aspects of blood vessel function, including contraction, cellular growth, and proliferation. Various physiological factors can selectively influence responses to a particular subtype, and the relative roles of each subtype can vary between vascular beds and along an individual blood vessel as its caliber changes. Functional studies in blood vessels suggest the presence of additional alpha-adrenoceptor subtypes not yet identified via molecular techniques. Optimization of the therapeutic profile of an alpha-adrenoceptor antagonist may be possible via enhancement of selectivity for a particular subtype or by design of a specific profile of affinity for the individual subtypes.

L8 ANSWER 51 OF 79 MEDLINE DUPLICATE 38
ACCESSION NUMBER: 96018842 MEDLINE
DOCUMENT NUMBER: 96018842 PubMed ID: 7565630
TITLE: Determination of structural domains for **G protein** coupling and ligand binding in beta 3-adrenergic receptor.
AUTHOR: Guan X M; Amend A; Strader C D
CORPORATE SOURCE: Department of Molecular Pharmacology and Biochemistry, Merck Research Labs, Rahway, New Jersey 07065, USA.
SOURCE: MOLECULAR PHARMACOLOGY, **(1995 Sep)** 48 (3) 492-8. Journal code: NGR; 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510

ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 20000303
Entered Medline: 19951030

AB The beta 3-adrenergic receptor (beta 3AR) is a member of the super-family of **G protein**-coupled receptors that are characterized by seven putative transmembrane helices connected by hydrophilic loops. The mechanism by which the activated beta ARs transmit the signals across the plasma membrane involves the stimulation of Gs, which in turn activates adenylyl cyclase, yielding the second messenger cAMP. In the present study, we created a series of mutant beta 3ARs to explore the structural basis for the subtype-specific binding of BRL 37344, a beta 3-selective agonist, and for the coupling of the receptor to Gs. To study the mechanism of subtype-specific binding of BRL 37344, **chimeric** beta 2/beta 3ARs were constructed and expressed in Raji cells. Binding studies suggest that the transmembrane segment 5 region of the beta 3AR contains critical determinants for observed high affinity for BRL 37344. Previous studies of beta 2ARs have demonstrated a role for the third **intracellular loop** in activating Gs. To investigate the role of this region in the beta 3AR, we constructed mutant beta 3ARs lacking a small segment of the amino- or carboxyl-terminal domain of the third **intracellular loop**. Expression of these mutant receptors in mouse L cells and Raji cells reveals that although both mutants are capable of binding the antagonist [125I]iodocyanopindolol, the agonist-stimulated cAMP production mediated by these mutant receptors is markedly attenuated or abolished. In addition, both mutant beta 3ARs exhibit an approximately 10-fold increase in affinity for agonist binding, whereas the affinity for antagonists is not affected. This increased agonist affinity is not altered by treatment with 100 microM 5' quanylyl-imidodiphosphate, suggesting that these mutant receptors are uncoupled from G proteins. The results of the present study demonstrate that these regions of the third **intracellular loop** of beta 3AR are critical for coupling to G proteins and suggest a role for these regions in maintaining the resting state of the unliganded receptor.

L8 ANSWER 52 OF 79 MEDLINE DUPLICATE 39
ACCESSION NUMBER: 95379793 MEDLINE
DOCUMENT NUMBER: 95379793 PubMed ID: 7651368
TITLE: Characterization of a **chimeric** human dopamine D3/D2 receptor functionally coupled to adenylyl cyclase in Chinese hamster ovary cells.
AUTHOR: Van Leeuwen D H; Eisenstein J; O'Malley K; MacKenzie R G
CORPORATE SOURCE: Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105, USA.
CONTRACT NUMBER: DA50651 (NIDA)
SOURCE: MOLECULAR PHARMACOLOGY, **(1995 Aug)** 48 (2) 344-51.
Journal code: NGR; 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19951005
Last Updated on STN: 19980206
Entered Medline: 19950926

AB Dopamine D3 receptor pharmacology differs from that of the dopamine D2 receptor despite a high degree of receptor sequence similarity. The greatest divergence of the primary sequences of D3 and D2 receptors occurs in the predicted third **intracellular loops** of the receptors, a region implicated in **G protein** binding and function. To determine whether this domain specifies the distinct ligand binding and signal transduction characteristics of the D3 receptor, we developed a **chimeric** receptor, replacing the third **intracellular loop** of the human D3 receptor with the third **intracellular loop** of the human D2 receptor. The

pharmacology of the **chimeric** receptor expressed in Chinese hamster ovary cells was examined and compared with that of human dopamine D2 and D3 receptors expressed in the same cell line. The **chimeric** receptor retained characteristic human D3 receptor binding; the D2 third **intracellular loop** present in the **chimeric** receptor did not reduce high affinity agonist binding, characteristic of the D3 receptor, or make high affinity sites sensitive to GTP analogs. Unlike the native human D3 receptor, the **chimeric** receptor was negatively coupled to adenylyl cyclase through a pertussis toxin-sensitive pathway, apparently mediated by the D2 third **intracellular loop**. The ability of D3 ligand binding domains to produce a D2 functional response implies that the third **intracellular loop** of the D3 receptor is unable to mediate this D2 response in Chinese hamster ovary cells. The inhibition of adenylyl cyclase seen with the **chimeric** receptor is less than the inhibition produced by D2 receptor coupling, suggesting that additional sequences in the D2 receptor contribute to normal **G protein** coupling.

L8 ANSWER 53 OF 79 MEDLINE
ACCESSION NUMBER: 95278590 MEDLINE
DOCUMENT NUMBER: 95278590 PubMed ID: 7758811
TITLE: The metabotropic glutamate receptors: their second **intracellular loop** plays a critical role in the **G-protein** coupling specificity.
AUTHOR: Pin J P; Gomeza J; Joly C; Bockaert J
CORPORATE SOURCE: Mecanismes Moleculaires des Communications Cellulaires, UPR-9023 CNRS, Montpellier, France.
SOURCE: BIOCHEMICAL SOCIETY TRANSACTIONS, **(1995 Feb)** 23 (1) 91-6.
Journal code: E48; 7506897. ISSN: 0300-5127.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950707
Last Updated on STN: 20000303
Entered Medline: 19950626

L8 ANSWER 54 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:59016 BIOSIS
DOCUMENT NUMBER: PREV199598073316
TITLE: **Chimeric** D1/D2 dopamine receptors: Distinct determinants of selective efficacy, potency, and signal transduction.
AUTHOR(S): Kozell, Laura B.; Machida, Curtis A.; Neve, Rachael L.; Neve, Kim A. (1)
CORPORATE SOURCE: (1) Res. Service, VA Med. Cent., 3710 SW US Veterans Hosp. Rd., Portland, OR 97201 USA
SOURCE: Journal of Biological Chemistry, (1994) Vol. 269, No. 48, pp. 30299-30306.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB D1/D2 **chimeras** were constructed that had D1 dopamine receptor sequence at the amino-terminal end and D2 dopamine receptor sequence at the carboxyl-terminal end. The **chimeras** with the first four, five and six transmembrane domains of the D1 receptor (CH2, CH3, CH4, respectively) bound the D1 receptor antagonist (3H)SCH 23390 with high affinity. Reciprocal **chimeras** constructed with D2 receptor sequence at the amino-terminal end displayed no detectable specific binding of (3H)SCH 23390, (125I)epidepride, or (3H)spiperone. CH2, CH3, and CH4 had lower affinity than either D1 or D2 dopamine receptors for the nonselective antagonists and agonists and D2-selective antagonists tested.
The **chimeric** receptors had affinities for three D1-selective

ligands and the D2-selective agonist, quinpirole, that were intermediate between D1 and D2 receptor affinities for the drug. The substantial loss or gain of affinity for three ligands upon replacement of D1 transmembrane VII with D2 sequence (CH4) suggests an important role for this region in the selectivity of these drugs. Stimulation of adenylyl cyclase activity by D1 agonists occurred in cells expressing CH3 and CH4, both of which included the D1 third **cytoplasmic loop**, but not in cells expressing CH1 or CH2, both with the D2 third **cytoplasmic loop**. However, only CH3 was able to mediate stimulation of adenylyl cyclase by quinpirole, implying that D2 receptor transmembrane domain VI was an important determinant of the selective efficacy of quinpirole. On the other hand, transmembrane domain VII was particularly important for the selective potency of quinpirole. Inhibition of beta-adrenergic receptor-stimulated adenylyl cyclase activity by dopamine was seen in cells expressing D2 receptors and CH1, but not CH2, CH3, or CH4. Thus, the third **cytoplasmic loop** of D1 dopamine receptors was crucial for the coupling of the receptors to G-s, but inhibition of adenylyl cyclase via G-i required structural features, such as the second **cytoplasmic loop** of the D2 receptor, in addition to the 3rd **cytoplasmic loop**.

L8 ANSWER 55 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:409008 BIOSIS
DOCUMENT NUMBER: PREV199497422008
TITLE: **Chimeric** muscarinic cholinergic:beta-adrenergic receptors that are functionally promiscuous among G proteins.
AUTHOR(S): Wong, Stephen K.-F.; Ross, Ellioitt M.
CORPORATE SOURCE: Dep. Pharmacol., Southwestern Graduate Sch. Biomedical Sci., Univ. Texas Southwestern Med. Cent., 5323 Harry Hines Blvd., Dallas, TX 75235-9041 USA
SOURCE: Journal of Biological Chemistry, (1994) Vol. 269, No. 29, pp. 18968-18976.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We evaluated the **G protein** selectivity of **chimeric** M1 and M2 muscarinic cholinergic receptors in which either the third intracellular (I3) loop or the N-terminal portion of this loop (the I3N peptide) was replaced by the corresponding sequence from the beta-1-adrenergic receptor. The **chimeras** retained agonist-dependent **G protein** regulatory activity, but were completely promiscuous among potential **G protein** targets. When expressed in transfected cells, the **chimeric** receptors activated adenylyl cyclase, the major target of the beta-adrenergic receptor, and activated phospholipase C via a pertussis toxin-insensitive **G protein**, presumably a G-q. G-s is not a target of either muscarinic receptor, and G-q is not a cellular target of either the M2 muscarinic or beta-adrenergic receptor. When co-reconstituted into phospholipid vesicles with purified G proteins, the **chimeric** receptors were completely nonselective among all G proteins tested. They activated G-i, G-o, G-z, G-q, and G-s with similar efficiencies. This promiscuity was largely suppressed, both in transfected cells and in reconstituted vesicles, by the additional replacement of the second intracellular (I2) loop of the beta-adrenergic receptor. Such double substitutions created receptors specific for G-s, the target of the beta-adrenergic receptor. These findings suggest that **G protein** specificity depends on the proper combination of multiple regions on a receptor's cytoplasmic surface. In addition, the promiscuous receptors described here may be useful for regulating novel G proteins whose natural regulators are not yet known.

L8 ANSWER 56 OF 79 MEDLINE DUPLICATE 40

ACCESSION NUMBER: 9[redacted]3043 MEDLINE
DOCUMENT NUMBER: 9[redacted]3043 PubMed ID: 8195123
TITLE: Gs regulation of endosome **fusion** suggests a role for signal transduction pathways in endocytosis.
AUTHOR: Colombo M I; Mayorga L S; Nishimoto I; Ross E M; Stahl P D
CORPORATE SOURCE: Department of Cell Biology and Physiology, Washington University, School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: AI20015 (NIAID)
GM42259 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1994 May 27)** 269 (21) 14919-23.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940707
Last Updated on STN: 20000303
Entered Medline: 19940629

AB Work from several laboratories indicates that guanine nucleotide-binding proteins (GTP-binding proteins) are required for intracellular vesicular transport. In a previous report we presented evidence indicating that one or more heterotrimeric G proteins regulate **fusion** between endosomes (Colombo, M. I., Mayorga, L. S., Casey, P. J., and Stahl, P. D. (1992) Science 255, 1695-1697). We now report on experiments showing that Gs plays a role in endosome **fusion**. We have used several reagents known to modulate Gs function including (i) peptides corresponding to the cytoplasmic domains of **G protein** -coupled receptors and peptides that mimic interaction of receptors with G proteins, (ii) anti-**G protein** antibodies, and (iii) cholera toxin. Synthetic peptides corresponding to the third **cytoplasmic loop** of the beta 2-adrenergic receptor which putatively interact with G alpha s inhibited endosomal **fusion**. The inhibitory effect of these peptides was prevented by a short preincubation of endosomes with guanosine-5'-3-O-(thio)triphosphate or by phosphorylating the peptide with cAMP-dependent protein kinase. The involvement of Gs in endosome recognition and/or the **fusion** process was assessed by testing an antibody against the COOH terminus of G alpha s. Anti-G alpha s IgG completely abolished **fusion** between endosomes. Lastly, preincubation of endosomal vesicles with cholera toxin abrogated **fusion** in the presence of NAD, whereas no effect was observed in the absence of the cofactor. Taken together these findings indicate a role for Gs in either the mechanism or the regulation of **fusion** among endosomes. These results raise the possibility that signal transduction through cytoplasmic domains of receptors may participate in the regulation of endocytic trafficking.

L8 ANSWER 57 OF 79 MEDLINE DUPLICATE 41
ACCESSION NUMBER: 94209338 MEDLINE
DOCUMENT NUMBER: 94209338 PubMed ID: 8157684
TITLE: Functional role of a cytoplasmic aromatic amino acid in muscarinic receptor-mediated activation of phospholipase C.
AUTHOR: Bluml K; Mutschler E; Wess J
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, NIDDKD, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1994 Apr 15)** 269 (15) 11537-41.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199405
ENTRY DATE: Entered STN: 19940526

Last Updated on STN: 20000303
Entered Medline: 19940519

AB The N-terminal portion of the third **intracellular loop** (i3) of muscarinic acetylcholine and other **G protein** -coupled receptors has been shown to largely determine the **G protein** coupling profile of a given receptor subtype. Using the rat m3 muscarinic receptor as a model system, we have recently demonstrated that a tyrosine residue (Tyr-254), located at the beginning of the i3 domain, is critically involved in muscarinic receptor-mediated stimulation of phosphatidylinositol (PI) hydrolysis (Bluml, K., Mutschler, E., and Wess, J. (1994) J. Biol. Chem. 269, 402-405). This study was designed to investigate the functional role of this amino acid in further molecular detail. Replacement of Tyr-254 (rat m3 receptor) with alanine or exchange of its position with Ile-253 virtually abolished receptor-mediated stimulation of PI hydrolysis studied in transfected COS-7 cells. In contrast, substitution of Tyr-254 by other aromatic residues such as phenylalanine or tryptophan resulted in mutant receptors that behaved functionally similar to the wild type m3 receptor. Introduction of Tyr-254 into the corresponding position (Ser-210) of the m2 muscarinic receptor (which is only poorly coupled to PI turnover) did not result in an enhanced PI response. However, "reinsertion" of Tyr-254 into a functionally inactive **chimeric** m3/m2 muscarinic receptor (containing m2 receptor sequence at the N terminus of the i3 loop) yielded a mutant receptor that was able to stimulate PI hydrolysis to a similar maximum extent as the wild type m3 receptor. Taken together, our data provide strong evidence that muscarinic receptor-mediated stimulation of PI metabolism is critically dependent on the presence and proper positioning of an aromatic residue at the beginning of the i3 loop.

L8 ANSWER 58 OF 79 MEDLINE DUPLICATE 42
ACCESSION NUMBER: 94344033 MEDLINE
DOCUMENT NUMBER: 94344033 PubMed ID: 8065262
TITLE: beta-Lactamase topology probe analysis of the OutO NMePhe peptidase, and six other Out protein components of the Erwinia carotovora general secretion pathway apparatus.
AUTHOR: Reeves P J; Douglas P; Salmond G P
CORPORATE SOURCE: Department of Biological Sciences, University of Warwick, Coventry, UK.
SOURCE: MOLECULAR MICROBIOLOGY, **(1994 May)** 12 (3) 445-57. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 20000303
Entered Medline: 19940920

AB The out gene cluster of Erwinia spp. encodes the proteins of the general secretory pathway (GSP) apparatus that is required for pectinase and cellulase secretion. We have used fusions between Erwinia carotovora subsp. carotovora (Ecc) out genes and the topology probe blaM to assess the ability of Out protein regions to export BlaM across the cytoplasmic membrane in Escherichia coli and Ecc. For the outO gene product (an NMePhe peptidase), **seven transmembrane** regions have been identified and one more is predicted. The region of OutO with the highest level of hydrophilicity is likely to exist as a large **cytoplasmic loop**, located between two hydrophobic domains, and is positioned towards the N-terminus of the protein. When BlaM was **fused** on the C-terminal side of the last hydrophobic stretch of OutO, the resulting hybrid protein transferred the BlaM moiety to the periplasm whilst retaining OutO activity. Removal of a portion of this hydrophobic stretch resulted in the loss of OutO activity, suggesting that there are tight constraints on the topological integrity of OutO for maintaining catalytic

function. When o[redacted]G, -H, -I, -J, -K and -N were f[redacted]d to blaM, the resulting ph[redacted]type suggested that the majori[redacted]of each protein was targeted to the periplasm. Our results indicate that these six Out proteins, when produced by E. coli or Ecc, each adopt, at least temporarily, a type II bitopic conformation in the cytoplasmic membrane. For OutG, -H, -I and -J this probably represents the membrane topology prior to processing by OutO in Ecc. When produced in vivo from a T7 gene 10 promoter construct, the outG product was processed in Ecc whereas the outO mutant RJP249 failed to process pre-OutG. BlaM fusions positioned on the C-terminal side of the hydrophobic stretches of pre-OutG, -H, -I, and -J were processed by wild-type Ecc but not RJP249 or E. coli DH1. Thus the periplasmic domains of these proteins play no role in the peptidase cleavage reaction. An OutG-BlaM **fusion** construct was used to demonstrate NMePhe peptidase activity in other bacterial strains including E. carotovora subsp. carotovora (ATCC39048), E. carotovora subsp. atroseptica (SCRI1043) and Erwinia chrysanthemi (3937).

L8 ANSWER 59 OF 79 MEDLINE DUPLICATE 43
ACCESSION NUMBER: 94147979 MEDLINE
DOCUMENT NUMBER: 94147979 PubMed ID: 8313879
TITLE: Domains involved in the specificity of **G protein** activation in phospholipase C-coupled metabotropic glutamate receptors.
AUTHOR: Pin J P; Joly C; Heinemann S F; Bockaert J
CORPORATE SOURCE: Centre CNRS-INSERM de Pharmacologie-Endocrinologie, Montpellier, France.
CONTRACT NUMBER: 1RO1 NS 28709 (NINDS)
NS11549 (NINDS)
SOURCE: EMBO JOURNAL, **(1994 Jan 15)** 13 (2) 342-8.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940330
Last Updated on STN: 20000303
Entered Medline: 19940321

AB **G protein**-coupled glutamate receptors (mGluR) have recently been characterized. These receptors have seven putative transmembrane domains, but display no sequence homology with the large family of **G protein**-coupled receptors. They constitute therefore a new family of receptors. Whereas mGluR1 and mGluR5 activate phospholipase C (PLC), mGluR2, mGluR3, mGluR4 and mGluR6 inhibit adenylyl cyclase (AC) activity. The third putative **intracellular loop**, which determines the **G protein** specificity in many **G protein**-coupled receptors, is highly conserved among mGluRs, and may therefore not be involved in the specific recognition of G proteins in this receptor family. By constructing **chimeric** receptors between the AC-coupled mGluR3 and the PLC-coupled mGluR1c, we report here that both the C-terminal end of the second **intracellular loop** and the segment located downstream of the seventh transmembrane domain are necessary for the specific activation of PLC by mGluR1c. These two segments are rich in basic residues and are likely to be amphipathic alpha-helices, two characteristics of the **G protein** interacting domains of all **G protein**-coupled receptors. This indicates that whereas no amino acid sequence homology between mGluRs and the other **G protein**-coupled receptors can be found, their **G protein** interacting domains have similar structural features.

L8 ANSWER 60 OF 79 MEDLINE DUPLICATE 44
ACCESSION NUMBER: 94103230 MEDLINE
DOCUMENT NUMBER: 94103230 PubMed ID: 8276814
TITLE: Domains of the human neutrophil N-formyl peptide receptor involved in **G protein** coupling. Mapping

with receptor-derived peptides.
AUTHOR: [redacted]reiber R E; Prossnitz E R; Ye R[redacted] Cochrane C G; Bokoch G M
CORPORATE SOURCE: Department of Cell Biology, Scripps Research Institute, La Jolla, California 92037.
CONTRACT NUMBER: GM39434 (NIGMS)
GM46572 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1994 Jan 7)** 269 (1) 326-31.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940218
Last Updated on STN: 20000303
Entered Medline: 19940208

AB Chemotactic signaling by the human neutrophil N-formyl peptide receptor requires its association with heterotrimeric **G protein** . Synthetic peptides and a **fusion** protein derived from the intracellular regions of the receptor were used to identify sites which interact with **G protein**. A peptide derived from the second **intracellular loop** (C12R), and peptides (F15R and S22L) and a **fusion** protein derived from the receptor's carboxyl terminus inhibited binding of anti-Gi alpha antibody (R16,17) to Gi alpha in a competitive enzyme-linked immunoassay, and antagonized pertussis-toxin catalyzed ADP-ribosylation of Gi alpha. C12R also inhibited **G protein**-dependent, high affinity ligand binding to the receptor and physical coupling of receptor to **G protein**. In contrast, a peptide consisting of the entire third loop of the N-formyl peptide receptor was totally inactive in these assays. Collectively, these data suggest that the second **intracellular loop** and the carboxyl-terminal tail are important for effective N-formyl peptide receptor/**G protein** coupling, but that the third **intracellular loop** is less important in coupling, unlike previous findings with other **G protein**-coupled receptor systems. The chemoattractant receptor family may rely on different structural determinants to interact with GTP-binding proteins.

L8 ANSWER 61 OF 79 MEDLINE DUPLICATE 45
ACCESSION NUMBER: 95094783 MEDLINE
DOCUMENT NUMBER: 95094783 PubMed ID: 8001544
TITLE: Activation of a GTP-binding protein and a GTP-binding-protein-coupled receptor kinase (beta-adrenergic-receptor kinase-1) by a muscarinic receptor m2 mutant lacking phosphorylation sites.
AUTHOR: Kameyama K; Haga K; Haga T; Moro O; Sadee W
CORPORATE SOURCE: Department of Biochemistry, Faculty of Medicine, University of Tokyo, Japan.
CONTRACT NUMBER: GM43102 (NIGMS)
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, **(1994 Dec 1)** 226 (2) 267-76.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 20000303
Entered Medline: 19950126

AB A mutant of the human muscarinic acetylcholine receptor m2 subtype (m2 receptor), lacking a large part of the third **intracellular loop**, was expressed and purified using the baculovirus/insect cell culture system. The mutant was not phosphorylated by beta-adrenergic-receptor kinase, as expected from the previous assignment of

phosphorylation [redacted]es to the central part of the [redacted]rd **intracellular lo[redacted]**. However, the m2 receptor muta[redacted]was capable of stimulating beta-adrenergic-receptor-kinase-1-mediated phosphorylation of a glutathione S-transferase **fusion** protein containing the m2 phosphorylation sites in an agonist-dependent manner. Both mutant and wild-type m2 receptors reconstituted with the guanine-nucleotide-binding regulatory proteins (**G protein**), G(o) and G(i)2, displayed guanine-nucleotide-sensitive high-affinity agonist binding, as assessed by displacement of [3H]quinuclidinyl-benzilate binding with carbamoylcholine, and both stimulated guanosine 5'-3-O-[35S]thiotriphosphate ([35S]GTP[S]) binding in the presence of carbamoylcholine and GDP. The Ki values of carbamoylcholine effects on [3H]quinuclidinyl-benzilate binding were indistinguishable for the mutant and wild-type m2 receptors. Moreover, the phosphorylation of the wild-type m2 receptor by beta-adrenergic-receptor kinase-1 did not affect m2 interaction with G proteins as assessed by the binding of [3H]quinuclidinyl benzilate or [35S]GTP[S]. These results indicate that (a) the m2 receptor serves both as an activator and as a substrate of beta-adrenergic-receptor kinase, and (b) a large part of the third **intracellular loop** of the m2 receptor does not contribute to interaction with G proteins and its phosphorylation by beta-adrenergic-receptor kinase does not uncouple the receptor and G proteins in reconstituted lipid vesicles.

L8 ANSWER 62 OF 79 MEDLINE DUPLICATE 46
ACCESSION NUMBER: 95047914 MEDLINE
DOCUMENT NUMBER: 95047914 PubMed ID: 7959413
TITLE: Regulation of **G protein**-coupled receptor kinase activity.
AUTHOR: Haga T; Haga K; Kameyama K; Nakata H
CORPORATE SOURCE: Institute for Brain Research, Faculty of Medicine, University of Tokyo, Japan.
SOURCE: NIPPON YAKURIGAKU ZASSHI. FOLIA PHARMACOLOGICA JAPONICA, **(1994 Sep)** 104 (3) 207-16. Ref: 50
Journal code: F2X; 0420550. ISSN: 0015-5691.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 20000303
Entered Medline: 19941214

AB Recent progress on the activation of **G protein**-coupled receptor kinases is reviewed. beta-Adrenergic receptor kinase (beta ARK) is activated by **G protein** beta gamma -subunits, which interact with the carboxyl terminal portion of beta ARK. Muscarinic receptor m2-subtypes are phosphorylated by beta ARK1 in the central part of the third **intracellular loop** (I3). Phosphorylation of I3-GST **fusion** protein by beta ARK1 is synergistically stimulated by the beta gamma -subunits and mastoparan or a peptide corresponding to portions adjacent to the transmembrane segments of m2-receptors or by beta gamma -subunits and the agonist-bound I3-deleted m2 variant. These results indicate that agonist-bound receptors serve as both substrates and activators of beta ARK.

L8 ANSWER 63 OF 79 MEDLINE DUPLICATE 47
ACCESSION NUMBER: 94326819 MEDLINE
DOCUMENT NUMBER: 94326819 PubMed ID: 8050479
TITLE: Activation of Gi protein by peptide structures of the muscarinic M2 receptor second **intracellular loop**.
AUTHOR: McClue S J; Baron B M; Harris B A
CORPORATE SOURCE: Marion Merrell Dow Research Institute, Strasbourg, France.

SOURCE: EUROPEAN JOURNAL OF PHARMACOLOGY, (1994 Apr 15)
(2) 185-93.
Journal code: EN6; 1254354. ISSN: 0014-2999.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19940914
Last Updated on STN: 20000303
Entered Medline: 19940906

AB The muscarinic M2 receptor that normally couples via Gi to inhibit adenylyl cyclase was made to couple to Gs by exchange of its third **intracellular loop** for the comparable domain of the beta 2-adrenoceptor. In HeLa cells transfected with the recombinant M2 beta i-3 cDNA, the chimaeric receptor showed carbachol-mediated activation of adenylyl cyclase (EC50 = 73 nM) that was blocked by atropine, but not by propranolol. The chimaeric receptor was shown to mediate a carbachol-stimulated, Bordetella pertussis toxin-sensitive GTPase activity in membranes of transfected HeLa cells. Interestingly, stimulation of adenylyl cyclase by carbachol was 2-fold higher in transfected cells that had been pretreated with pertussis toxin. These data suggested that the M2 beta i-3 receptor was able to couple to both Gi and Gs, and that the ability to recognise and stimulate Gi did not involve the third **cytoplasmic loop** of M2. We investigated peptide elements taken from the second **intracellular loop** of the M2 receptor for their ability to mediate activation of Gi and found that a nine amino acid peptide representing the C-terminal sequence, VKRTTKMAG-NH2 (V9G), was capable of inhibiting forskolin-stimulated adenylyl cyclase by up to 18% and could stimulate high affinity GTPase activity of rat brain membranes by 32%. Further, V9G was shown to cause a doubling of the initial rate of [35S]GTP gamma S binding to purified bovine brain Gi/Go in reconstituted phospholipid vesicles. These data identify a domain on the second **intracellular loop** of the muscarinic M2 receptor that is involved in the selection of a pertussis toxin-sensitive **G protein**.

L8 ANSWER 64 OF 79 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:274901 BIOSIS
DOCUMENT NUMBER: PREV199497287901
TITLE: Activation of G-i protein by peptide structures of the muscarinic M-2 receptor second **intracellular loop**.
AUTHOR(S): McClue, Steven J.; Baron, Bruce M.; Harris, Bruce A. (1)
CORPORATE SOURCE: (1) Marion Merrell Dow Res. Inst., 2110 E. Galbraith Road, Cincinnati, OH 45205-6290 USA
SOURCE: European Journal of Pharmacology Molecular Pharmacology Section, (1994) Vol. 15, No. 2, pp. 185-193.
ISSN: 0922-4106.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The muscarinic M-2 receptor that normally couples via G-i to inhibit adenylyl cyclase was made to couple to G-s by exchange of its third **intracellular loop** for the comparable domain of the beta-2-adrenoceptor. In HeLa cells transfected with the recombinant M-2-beta-i-3 cDNA, the **chimeric** receptor showed carbachol-mediated activation of adenylyl cyclase (EC-50 = 73 nM) that was blocked by atropine, but not by propranolol. The **chimeric** receptor was shown to mediate a carbachol-stimulated, Bordetella pertussis toxin-sensitive GTPase activity in membranes of transfected HeLa cells. Interestingly, stimulation of adenylyl cyclase by carbachol was 2-fold higher in transfected cells that had been pretreated with pertussis toxin.
These data suggested that the M-2-beta-i-3 receptor was able to couple to

both G-i and G- and that the ability to recogn and stimulate G-i did not involve the ird **cytoplasmic loop** of M-2. W investigated peptide elements taken from the second **intracellular loop** of the M-2 receptor for their ability to mediate activation of G-i and found that a nine amino acid peptide representing the C-terminal sequence, VKRTTKMAG-NH-2 (V9G), was capable of inhibiting forskolin-stimulated adenylyl cyclase by up to 18% and could stimulate high affinity GTPase activity of rat brain membranes by 32%. Further, V9G was shown to cause a doubling of the initial rate of (35S)GTP-gamma-S binding to purified bovine brain G-i/G-o in reconstituted phospholipid vesicles. These data identify a domain on the second **intracellular loop** of the muscarinic M-2 receptor that is involved in the selection of a pertussis toxin-sensitive **G protein**.

L8 ANSWER 65 OF 79 MEDLINE DUPLICATE 48
ACCESSION NUMBER: 93346382 MEDLINE
DOCUMENT NUMBER: 93346382 PubMed ID: 8393861
TITLE: Functional nonequivalence of structurally homologous domains of neurokinin-1 and neurokinin-2 type tachykinin receptors.
AUTHOR: Blount P; Krause J E
CORPORATE SOURCE: Department of Anatomy and Neurobiology, Washington University School of Medicine, Saint Louis, Missouri 63110.
CONTRACT NUMBER: NS07129 (NINDS)
NS21937 (NINDS)
NS29343 (NINDS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1993 Aug 5)** 268 (22) 16388-95.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19930924
Last Updated on STN: 19970203
Entered Medline: 19930907

AB The neurokinin-1 (NK-1) and neurokinin-2 (NK-2) receptors are both members of the tachykinin receptor family. Although both receptors bind peptide ligands synthesized from common precursors and activate inositol 1,4,5-triphosphate and cAMP responses, differences between these receptors have been observed in the extent and kinetics of agonist-induced responses. Here, to test if structurally homologous domains of the NK-1 and NK-2 receptors are functionally distinct, stably transfected Chinese hamster ovary (CHO) cell lines expressing receptors that had either their putative third **cytoplasmic loop** (C3) or carboxyl tail (CT) domains replaced with the equivalent domain of the other receptor were compared with stably transfected CHO cell lines expressing wild-type receptors. Radioligand binding demonstrated that each of these **chimeric** receptors had agonist binding affinities indistinguishable from wild-type receptors. However, not all **chimeric** receptors were equivalent in their ability to stimulate inositol phospholipid turnover and cAMP production. The data suggest that the NK-1 C3 and the NK-2 CT domains play important roles in **G-protein** activation that cannot be replaced by the analogous domain of the other receptor. The characterization of CHO cell lines expressing truncated forms of both receptors supported the hypothesis that the CT domain of the NK-2, but not the NK-1, receptor plays a critical role in **G-protein** activation. The data suggest a potential mechanism for the differences observed in response characteristics in tissues expressing NK-1 and NK-2 receptors and demonstrate that the mechanisms whereby highly homologous receptors activate G-proteins can be different.

L8 ANSWER 66 OF 79 MEDLINE
ACCESSION NUMBER: 94052203 MEDLINE

DOCUMENT NUMBER: [redacted]52203 PubMed ID: 8234336
TITLE: [redacted]ations that alter the third **cy[redacted]lasmic loop** of the a-factor receptor lead to a constitutive and hypersensitive phenotype.
AUTHOR: Boone C; Davis N G; Sprague G F Jr
CORPORATE SOURCE: Institute of Molecular Biology, University of Oregon, Eugene 97403.
CONTRACT NUMBER: GM12672 (NIGMS)
GM38157 (NIGMS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1993 Nov 1)** 90 (21) 9921-5.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19970203
Entered Medline: 19931209

AB The STE3 gene of Saccharomyces cerevisiae encodes a **G protein**-coupled receptor that is specific for the mating pheromone a-factor. The ste3L194Q mutation, which leads to the substitution of glutamine for leucine-194 within the third **cytoplasmic loop** of the receptor, resulted in a 20-fold increase in pheromone sensitivity and also caused partial constitutive activation of the response pathway. Moreover, other amino acid substitutions at the 194 position and several deletion mutations that collectively remove most of the third **cytoplasmic loop** resulted in hyperactive receptors. Therefore, we suggest that one role of the third **cytoplasmic loop** is to function as a negative regulatory domain involved in the maintenance of a nonsignaling state of the receptor. The constitutive activity and the pheromone hypersensitivity of ste3L194Q cells were recessive, suggesting that the wild-type receptor can antagonize the signal associated with the activated receptor. The ste3 delta 306 mutation, which results in truncation of most of the C-terminal domain of the receptor, led to a 20-fold increase in pheromone sensitivity, indicating that this domain also mediates negative regulation of the receptor. The ste3L194Q and ste3 delta 306 mutations appear to affect receptor activity independently, because the double mutant was associated with a 400-fold increase in pheromone sensitivity.

L8 ANSWER 67 OF 79 MEDLINE DUPLICATE 49
ACCESSION NUMBER: 93219433 MEDLINE
DOCUMENT NUMBER: 93219433 PubMed ID: 8385357
TITLE: Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular "cross-talk" between **G-protein**-linked receptors.
AUTHOR: Maggio R; Vogel Z; Wess J
CORPORATE SOURCE: Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1993 Apr 1)** 90 (7) 3103-7.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930521
Last Updated on STN: 20000303
Entered Medline: 19930504

AB We have tested the hypothesis that guanine-nucleotide-binding-protein-coupled receptors may be able to interact with each other at a molecular level. To address this question, we have initially created two

chimeric receptors, alpha 2/m3 and m3/alpha 2, in which the C-terminal receptor portions (containing transmembrane domains VI and VII) were exchanged between the alpha 2C-adrenergic and the m3 muscarinic receptor. Transfection of COS-7 cells with either of the two **chimeric** constructs alone did not result in any detectable binding activity for the muscarinic ligand N-[3H]methylscopolamine or the adrenergic ligand [3H]rauwolscine. However, cotransfection with alpha 2/m3 and m3/alpha 2 resulted in the appearance of specific binding sites (30-35 fmol/mg of membrane protein) for both radioligands. These sites displayed ligand binding properties similar to those of the two wild-type receptors. Furthermore, COS-7 cells cotransfected with alpha 2/m3 and m3/alpha 2 were able to mediate a pronounced stimulation of phosphatidylinositol hydrolysis upon stimulation with the muscarinic agonist carbachol (Emax approximately 40-50% of wild-type m3). A mutant m3 receptor (containing 16 amino acids of m2 receptor sequence at the N terminus of the third **cytoplasmic loop**) that was capable of binding muscarinic ligands but was virtually unable to stimulate phosphatidylinositol hydrolysis was also used in various cotransfection experiments. Coexpression of this **chimeric** receptor with other functionally impaired mutant muscarinic receptors (e.g., with an m3 receptor containing a Pro-->Ala point mutation in transmembrane region VII) resulted in a considerable stimulation of phosphatidylinositol breakdown after carbachol treatment (Emax approximately 40-50% of wild-type m3). Thus, these data suggest that guanine-nucleotide-binding-protein-coupled receptors can interact with each other at a molecular level. One may speculate that the formation of receptor dimers involving the intermolecular exchange of N- and C-terminal receptor domains (containing transmembrane domains I-V and VI and VII, respectively) may underlie this phenomenon.

L8 ANSWER 68 OF 79 MEDLINE DUPLICATE 50

ACCESSION NUMBER: 93260458 MEDLINE
DOCUMENT NUMBER: 93260458 PubMed ID: 8098355
TITLE: Development of polyclonal anti-D2 dopamine receptor antibodies to **fusion** proteins: inhibition of D2 receptor-**G protein** interaction.
AUTHOR: Boundy V A; Luedtke R R; Molinoff P B
CORPORATE SOURCE: Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia 19104-6084.
CONTRACT NUMBER: MH14654 (NIMH)
NS18591 (NINDS)
SOURCE: JOURNAL OF NEUROCHEMISTRY, **(1993 Jun)** 60 (6) 2181-91.
Journal code: JAV; 2985190R. ISSN: 0022-3042.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930625
Last Updated on STN: 20000303
Entered Medline: 19930615

AB Portions of the cDNA encoding the third **intracellular loop** (i3 loop) of the long and short isoforms of the rat D2 dopamine receptor were subcloned into the vector pNMHUBpoly and expressed in Escherichia coli as **fusion** proteins. The **fusion** proteins were gel-purified and used to immunize rabbits for the production of polyclonal anti-receptor antisera. The anti-**fusion** protein antisera recognized synthetic peptides corresponding to segments of the i3 loops of D2 dopamine receptors in a solid-phase radioimmunoassay. Antisera

were tested in a immunoprecipitation assay using the reversible D2 antagonist [125I]NCQ 298 and digitonin-solubilized extracts of canine and rat caudate. [125I]-NCQ 298 bound reversibly and with high affinity (KD = 0.14 nM) to receptors in solubilized extracts enriched by chromatography on heparin-agarose. The anti-UBI-D2i3L and anti-UBI-D2i3s antisera were able to immunoprecipitate quantitatively D2 dopamine receptors labeled with [125I]NCQ 298 from solubilized rat caudate. The antibodies were tested for their ability to affect the coupling of D2 dopamine receptors to GTP-binding proteins in digitonin-solubilized rat caudate. Both anti-UBI-D2i3L and anti-UBI-Di3s antisera were able to inhibit the high-affinity binding of the agonist N-propylnorapomorphine to digitonin-solubilized rat caudate. These findings indicate that the i3 loop of the D2 dopamine receptor is an important determinant for coupling of the **G protein**.

L8 ANSWER 69 OF 79 MEDLINE
ACCESSION NUMBER: 93197892 MEDLINE
DOCUMENT NUMBER: 93197892 PubMed ID: 8383880
TITLE: Antagonism of catecholamine receptor signaling by expression of cytoplasmic domains of the receptors.
AUTHOR: Luttrell L M; Ostrowski J; Cotecchia S; Kendall H; Lefkowitz R J
CORPORATE SOURCE: Department of Medicine, Duke University Medical Center, Durham, NC 27710.
CONTRACT NUMBER: HL16037 (NHLBI)
SOURCE: SCIENCE, **(1993 Mar 5)** 259 (5100) 1453-7.
Journal code: UJ7; 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930423
Last Updated on STN: 20000303
Entered Medline: 19930409

AB The actions of many hormones and neurotransmitters are mediated by the members of a superfamily of receptors coupled to heterotrimeric guanine nucleotide-binding proteins (G proteins). These receptors are characterized by a highly conserved topographical arrangement in which **seven transmembrane** domains are connected by intracellular and extracellular loops. The interaction between these receptors and G proteins is mediated in large part by the third **intracellular loop** of the receptor. Coexpression of the third **intracellular loop** of the alpha 1B-adrenergic receptor with its parent receptor inhibited receptor-mediated activation of phospholipase C. The inhibition extended to the closely related alpha 1C-adrenergic receptor subtype, but not the phospholipase C-coupled M1 muscarinic acetylcholine receptor nor the adenylate cyclase-coupled D1A dopamine receptor. These results suggest that the receptor-**G protein** interface may represent a target for receptor antagonist drugs.

L8 ANSWER 70 OF 79 MEDLINE
ACCESSION NUMBER: 94158903 MEDLINE
DOCUMENT NUMBER: 94158903 PubMed ID: 8114758
TITLE: Positive charges in a putative amphiphilic helix in the carboxyl-terminal region of the third **intracellular loop** of the luteinizing hormone/chorionic gonadotropin receptor are not required for hormone-stimulated cAMP production but are necessary for expression of the receptor at the plasma membrane.
AUTHOR: Wang H; Jaquette J; Collison K; Segaloff D L
CORPORATE SOURCE: Department of Physiology and Biophysics, University of Iowa
College of Medicine, Iowa City 52242.
CONTRACT NUMBER: DK-25295 (NIDDK)
HD-00968 (NICHD)
HD22196 (NICHD)
SOURCE: MOLECULAR ENDOCRINOLOGY, **(1993 Nov)** 7 (11)

7-44.
rnal code: NGZ; 8801431. ISSN: 88-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940406
Last Updated on STN: 20000303
Entered Medline: 19940328

AB The LH/CG receptor (LHR) is a member of the family of **G protein**-coupled receptors and activates Gs when stimulated by LH or CG. Studies from other **G protein**-coupled receptors have implicated the carboxyl-terminal region of the third **intracellular loop** as being involved in the activation of G proteins. It has been suggested that the potential ability of this region to form an amphiphilic helix, with positively charged residues aligned to one face, may be important for this biological activity. To test whether the positively charged lysine residues, and thus an amphiphilic helix, in the carboxyl terminal region of the rat LHR (rLHR) are indeed important in the activation of Gs by the rLHR, a mutant rLHR was constructed in which lysines 541, 544, and 557 were simultaneously substituted with alanines. Clonal 293 cells expressing comparable numbers of cell-surface recombinant wild type rLHR or rLHR(K541,544,547A) were generated. Cells expressing the mutant receptor-bound human CG (hCG) with the same high affinity as those expressing the wild type rLHR. Since the numbers of receptors and binding affinities between the two cell lines were comparable, any changes in basal or hCG stimulated cAMP production could readily be interpreted as an alteration in the mutant receptor's ability to activate Gs. It was found, however, that basal cAMP production, the concentration of hCG required to elicit half-maximal cAMP production, and the maximal levels of cAMP produced in response to hCG were all unchanged in cells expressing rLHR(K541,544,547A).(ABSTRACT TRUNCATED AT 250 WORDS)

L8 ANSWER 71 OF 79 MEDLINE
ACCESSION NUMBER: 94166426 MEDLINE
DOCUMENT NUMBER: 94166426 PubMed ID: 7509921
TITLE: Functional domains of human endothelin receptor.
AUTHOR: Adachi M; Hashido K; Trzeciak A; Watanabe T; Furuichi Y; Miyamoto C
CORPORATE SOURCE: Department of Molecular Genetics, Nippon Roche Research Center, Kamakura, Japan.
SOURCE: JOURNAL OF CARDIOVASCULAR PHARMACOLOGY, **(1993)** 22 Suppl 8 S121-4.
Journal code: K78; 7902492. ISSN: 0160-2446.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940412
Last Updated on STN: 20000303
Entered Medline: 19940404

AB The ligand binding site to the ETA receptor was investigated by substitution of each 5-amino acid sequence located in the second extracellular (B) region of the ETA receptor with the cognate sequences of the beta 2-adrenergic receptor. A 5-amino acid sequence (140-KLLAG-144) in the B-loop region was implicated as the most important element required for ligand binding. In addition, both the third and the fourth extracellular regions (C- and D-loops), including the flanking transmembrane regions, were found to play an important role in ligand selection. As for the biological significance of the intracellular regions of the ETA receptor, we have found that the C-terminal 8-amino acid residues located in close proximity to the seventh transmembrane region

and the C-termin[redacted] 16-amino acid residues in the [redacted]rd **intracellular lo**[redacted] are important for the binding [redacted] ET-1. Therefore, the intracellular third loop and C-terminal domains seem to contribute to the three-dimensional structure of the ligand binding site located in the extracellular domains. The same lines of experiment showed that the ETA receptor requires > 13 amino acid residues at the proximal cytoplasmic tail and 10 amino acid residues in the C-terminal region of the third **intracellular loop** to induce an ET-1-dependent increase in [Ca2+]i. Both regions are possibly involved in the interaction with **G-protein**.

L8 ANSWER 72 OF 79 MEDLINE
ACCESSION NUMBER: 93188868 MEDLINE
DOCUMENT NUMBER: 93188868 PubMed ID: 8383288
TITLE: Mutation of a highly conserved acidic residue present in the second **intracellular loop** of **G-protein**-coupled receptors does not impair hormone binding or signal transduction of the luteinizing hormone/chorionic gonadotropin receptor.
AUTHOR: Wang Z; Wang H; Ascoli M
CORPORATE SOURCE: Department of Pharmacology, University of Iowa College of Medicine, Iowa City 52242-1109.
CONTRACT NUMBER: CA-40629 (NCI)
DK-25295 (NIDDK)
SOURCE: MOLECULAR ENDOCRINOLOGY, **(1993 Jan)** 7 (1) 85-93.
Journal code: NGZ; 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930416
Last Updated on STN: 20000303
Entered Medline: 19930406

AB Sequence alignment shows that there is a highly conserved acidic residue (D or E) at the boundary between the third transmembrane domain and the second **intracellular loop** of the superfamily of **G-protein**-coupled receptors. Previous mutagenesis studies demonstrated that substitution of this acidic residue in the beta 2-adrenergic, muscarinic m1, and alpha 2A-adrenergic receptors by the corresponding amide preserved high affinity agonist binding, but significantly reduced or completely abolished activation of the respective effector. To determine whether the corresponding amino acid residue (E441) played a similar role in the functions of the rat LH/CG receptor, we used site-directed mutagenesis to substitute it by D or Q. The wild-type and mutant receptors (E441D or E441Q) were then transfected into human embryonic kidney 293 cells and tested for their ability to bind hCG and respond to it with increased cAMP accumulation. As predicted, the mutant LH/CG receptors were found to bind hCG with high affinity. In contrast to the results summarized above, however, an E441Q or an E441D mutation in the LH/CG receptor results in only a slight increase in the EC50 for cAMP accumulation without decreasing the maximal response attained. The most remarkable effect of these mutations was on localization of the receptor. Thus, while most of the receptors expressed in cells transfected with the E441D mutant could be detected by measuring hormone binding to intact cells, most of the receptors expressed in cells transfected with the E441Q mutant could be detected only upon solubilization of the cells with detergent.

L8 ANSWER 73 OF 79 MEDLINE
ACCESSION NUMBER: 94115367 MEDLINE
DOCUMENT NUMBER: 94115367 PubMed ID: 8287069
TITLE: Identification of specific intracellular domains of the human ETA receptor required for ligand binding and signal transduction.
AUTHOR: Hashido K; Adachi M; Gamou T; Watanabe T; Furuichi Y;

CORPORATE SOURCE: [redacted]amoto C
[redacted]artment of Molecular Genetics, [redacted]ppon Roche Research Center, Kamakura, Japan.
SOURCE: CELLULAR AND MOLECULAR BIOLOGY RESEARCH, **(1993)** 39 (1) 3-12.
Journal code: BSK; 9316986. ISSN: 0968-8773.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940312
Last Updated on STN: 19970203
Entered Medline: 19940218

AB We have investigated the function of the C-terminal and the third intracellular domains of the ETA receptor by expressing truncated and mutated ETA receptors in COS-7 and CHO cells. All the C-terminal truncated ETA receptors were produced at a similar expression level and were detected in the cell membrane using indirect immunostaining. The sizes of the truncated ETA receptors were decreased in proportion to the molecular mass of the truncated amino acid sequence. When the ligand binding activities were determined for various truncated ETA receptors, it was found that more than eight amino acid residues at the proximal cytoplasmic tail of the ETA receptor were required for ET-1 binding. In addition, the deletion of 16 C-terminal amino acid residues from the third **intracellular loop** severely decreased the ligand binding activity. It seems that deletion of these cytoplasmic domains of the ETA receptor influences the three-dimensional structure of the ligand binding site located in the extracellular domains. The ETA receptor required more than 13 amino acid residues in the proximity of C-terminal cytoplasmic tail and 10 amino acid residues in the C-terminal region of the third **intracellular loop** to induce the ET-1 dependent increase in intracellular calcium concentration. Both regions are possibly coupled with **G-protein** to transmit the ET-1 signal.

L8 ANSWER 74 OF 79 MEDLINE DUPLICATE 51
ACCESSION NUMBER: 92112880 MEDLINE
DOCUMENT NUMBER: 92112880 PubMed ID: 1309789
TITLE: Discrete amino acid sequences of the alpha 1-adrenergic receptor determine the selectivity of coupling to phosphatidylinositol hydrolysis.
AUTHOR: Cotecchia S; Ostrowski J; Kjelsberg M A; Caron M G; Lefkowitz R J
CORPORATE SOURCE: Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710.
CONTRACT NUMBER: HL16037 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1992 Jan 25)** 267 (3) 1633-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 19980206
Entered Medline: 19920218

AB We have constructed a variety of **chimeric** beta 2/alpha 1 adrenergic receptors (AR) in which selected portions of the third **intracellular loop** of the alpha (1B)AR were substituted into the corresponding regions of the beta 2AR. The mutant receptors were both transiently and permanently expressed in COS-7 or L-cells, respectively, and tested for their ability to mediate epinephrine-induced activation of polyphosphoinositide (PI) hydrolysis and adenylylcyclase. We have determined that 27 amino acids of the alpha (1B)AR (residues 233-259)

derived from th -terminal portion of the third **tracellular loop** represent structural determinant confer g to the beta 2AR the ability to activate PI hydrolysis. This finding suggests that in the alpha (1B)AR the N-terminal portion of the third **intracellular loop** plays a major role in determining the selectivity of receptor-**G protein** coupling. However, replacement of alpha 1B sequences in the third **intracellular loop** of the beta 2AR did not abolish the latter receptor's coupling to activation of adenylylcyclase, thus resulting in **chimeric** adrenergic receptors which activated both PI hydrolysis and adenylylcyclase. These results indicate that, even if the N-terminal portion of the third **intracellular loop** is a major determinant of the selectivity of receptor-**G protein** coupling, other structural domains of the receptors also modulate this property. The comparison of the amino acid sequences which determine the selectivity of **G protein** coupling in functionally similar receptors may help to elucidate the structural basis for activation of specific **G protein**-effector systems.

L8 ANSWER 75 OF 79 MEDLINE
ACCESSION NUMBER: 92357031 MEDLINE
DOCUMENT NUMBER: 92357031 PubMed ID: 1323056
TITLE: Structural subtypes of the dopamine D2 receptor are functionally distinct: expression of the cloned D2A and D2B subtypes in a heterologous cell line.
AUTHOR: Hayes G; Biden T J; Selbie L A; Shine J
CORPORATE SOURCE: Garvan Institute of Medical Research, Sydney, Australia.
SOURCE: MOLECULAR ENDOCRINOLOGY, **(1992 Jun)** 6 (6) 920-6.
Journal code: NGZ; 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19920925
Last Updated on STN: 20000303
Entered Medline: 19920904

AB Dopamine, a major neurotransmitter in the mammalian nervous system, exerts its physiological effects through receptors of the **G-protein**-coupled receptor superfamily. Two major classes of dopamine receptor, D1 and D2, are distinguishable by both biochemical and pharmacological criteria. D1 receptors activate adenylyl cyclase, whereas the D2 class of receptors inhibits this second messenger system. Two subtypes of the human dopamine D2 receptor are generated by alternate splicing of the RNA transcript of a single gene. These two forms, termed D2A (long) and D2B (short), differ by the insertion of 29 amino acids within the putative third **cytoplasmic loop**, an intracellular domain thought to have a role in coupling this class of receptors to particular second messenger systems. We report here that the D2A and D2B structural subtypes are also functionally distinct. Expression of the two subtypes in a fibroblast cell line revealed that while occupation of both receptors leads to an increase in cytosolic free calcium concentration, they differ in their capacity to inhibit cAMP production. At physiological dopamine concentrations, the D2B-mediated inhibition of calcitonin gene-related peptide-stimulated cAMP accumulation is almost double the response mediated by the D2A subtype. Furthermore, the D2B subtype can maximally attenuate cAMP accumulation by up to 85%, whereas the D2A subtype is less effective, maximally inhibiting cAMP accumulation by only 64%. The D2A and D2B subtypes, thus, constitute functionally distinct forms of the dopamine receptor that can couple to multiple intracellular signalling pathways.

L8 ANSWER 76 OF 79 MEDLINE DUPLICATE 52
ACCESSION NUMBER: 92115690 MEDLINE
DOCUMENT NUMBER: 92115690 PubMed ID: 1731321

TITLE: [redacted]carinic receptor-operated Ca2+ [redacted]flux in transfected [redacted]roblast cells is independent o[redacted]nositol phosphates and release of intracellular Ca2+.
AUTHOR: Felder C C; Poulter M O; Wess J
CORPORATE SOURCE: Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1992 Jan 15)** 89 (2) 509-13.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 20000303
Entered Medline: 19920218

AB Receptor-mediated changes in cytoplasmic calcium concentrations occur either through release from intracellular calcium stores or by the opening of channels in the plasma membrane, allowing influx of calcium from the extracellular fluid. Carbachol, a muscarinic receptor agonist, stimulated both calcium influx and inositol 1,4,5-trisphosphate (InsP3)-mediated intracellular calcium release in A9 fibroblast cells expressing a m3 muscarinic receptor clone. The calcium influx persisted even after pretreatment of cells with phorbol 12-myristate 13-acetate, which completely prevented the rise in inositol phosphates and intracellular calcium levels. The calcium influx was blocked by divalent cations but was not affected by inhibitors of voltage-dependent calcium channels or high potassium depolarization, indicating the presence of a receptor-operated and voltage-insensitive calcium channel in these cells. Calcium influx was not stimulated by the addition of cAMP analogs or arachidonic acid. To examine the possible involvement of G proteins in m3 receptor-activated calcium influx, two **chimeric** m2 and m3 muscarinic receptors were expressed in A9 cells in which the third **cytoplasmic loop** (the primary structural determinant in **G protein** coupling selectivity of muscarinic receptors) had been exchanged between the m2 receptor, which has no effect on calcium influx, and the m3 receptor. Calcium influx was found to be associated with a structural component of the m3 muscarinic receptor other than the third **cytoplasmic loop**.

L8 ANSWER 77 OF 79 MEDLINE DUPLICATE 53
ACCESSION NUMBER: 91138769 MEDLINE
DOCUMENT NUMBER: 91138769 PubMed ID: 1995348
TITLE: A **chimeric** D2 dopamine/m1 muscarinic receptor with D2 binding specificity mobilizes intracellular calcium in response to dopamine.
AUTHOR: England B P; Ackerman M S; Barrett R W
CORPORATE SOURCE: Affymax Research Institute, Palo Alto, CA 94304.
SOURCE: FEBS LETTERS, **(1991 Feb 11)** 279 (1) 87-90.
Journal code: EUH; 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910412
Last Updated on STN: 19910412
Entered Medline: 19910327

AB Using PCR methodology, a **chimeric** receptor cDNA was constructed in which the entire third **cytoplasmic loop** of the human D2 dopamine receptor was replaced by the analogous portion of the human m1 muscarinic receptor. When expressed in CHO cells, the **chimeric** D2/m1 receptor bound dopaminergic ligands with affinities

similar to the native D2(414) receptor. Intracellular calcium levels (measured with fura-2) were not altered when CHO cells expressing the D2(414) receptor were exposed to dopamine. In contrast, dopamine elevated intracellular calcium levels in cells expressing the D2/m1 **chimeric** receptor in a dose-dependent manner which was blocked by the D2 antagonist, fluphenazine. The ability to construct **G-protein**-linked receptor **chimeras** which mobilize calcium with nearly unaltered pharmacologic specificity raises the possibility of a generic strategy for creating non-radioisotopic reporter systems for use in drug discovery.

L8 ANSWER 78 OF 79 MEDLINE DUPLICATE 54
ACCESSION NUMBER: 90202894 MEDLINE
DOCUMENT NUMBER: 90202894 PubMed ID: 2156845
TITLE: **Chimeric** muscarinic cholinergic: beta-adrenergic receptors that activate Gs in response to muscarinic agonists.
AUTHOR: Wong S K; Parker E M; Ross E M
CORPORATE SOURCE: Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas 75235-9041.
CONTRACT NUMBER: GM11943 (NIGMS)
GM30355 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1990 Apr 15)** 265 (11) 6219-24.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 20000303
Entered Medline: 19900510

AB The M1-muscarinic cholinergic receptor (M1AChR) stimulates the release of inositol phosphates (IPs) but does not activate adenylyl cyclase. The beta-adrenergic receptor (beta-AR) stimulates adenylyl cyclase but has no effect on IP release. Amino acid sequences corresponding to the second (I2) and third (I3) **intracellular loops** of the turkey erythrocyte beta-AR and a 12-amino acid segment near the N-terminal end of the I3 region were substituted into the corresponding regions of the human M1AChR. **Chimeric** receptors that contained either the entire I3 loop or the N-terminal dodecapeptide of that loop both mediated the 2-4-fold stimulation of adenylyl cyclase activity in membrane fractions of COS, A293, or Sf9 cells in response to carbachol. These **chimeric** receptors also retained the ability to stimulate IP release to the same extent as did the M1AChR. In COS cells transfected with the I3 **chimeric** receptor, the EC50 for carbachol was approximately 7 microM for the stimulation of adenylyl cyclase and approximately 2 microM for the release of IP; M1AChR-mediated IP release displayed an EC50 of approximately 0.2 microM. Substitution of the I2 region of the beta-AR into the M1AChR did not by itself alter selectivity for signaling. However, the I2+I3 and I2+dodecapeptide combined replacements stimulated adenylyl cyclase fully and caused at most 25% of the maximal stimulation of IP release observed with the M1AChR. Thus, a small region in the third **cytoplasmic loop** can alter the G proteins to which a receptor is coupled, but interaction among loops is evidently involved in fully determining **G protein** selectivity.

L8 ANSWER 79 OF 79 MEDLINE DUPLICATE 55
ACCESSION NUMBER: 90222137 MEDLINE
DOCUMENT NUMBER: 90222137 PubMed ID: 2158097
TITLE: Regions of the alpha 1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function.
AUTHOR: Cotecchia S; Exum S; Caron M G; Lefkowitz R J

CORPORATE SOURCE: [redacted]artment of Medicine, Howard Hu[redacted]s Medical Institute, [redacted]e University Medical Center, D[redacted]am, NC 27710.
CONTRACT NUMBER: HL16037 (NHLBI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1990 Apr)** 87 (8) 2896-900.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900622
Last Updated on STN: 19970203
Entered Medline: 19900524

AB Regions of the hamster alpha 1-adrenergic receptor (alpha 1 AR) that are important in GTP-binding protein (**G protein**)-mediated activation of phospholipase C were determined by studying the biological functions of mutant receptors constructed by recombinant DNA techniques. A **chimeric** receptor consisting of the beta 2-adrenergic receptor (beta 2AR) into which the putative third **cytoplasmic loop** of the alpha 1AR had been placed activated phosphatidylinositol metabolism as effectively as the native alpha 1AR, as did a truncated alpha 1AR lacking the last 47 residues in its cytoplasmic tail. Substitutions of beta 2AR amino acid sequence in the intermediate portions of the third **cytoplasmic loop** of the alpha 1AR or at the N-terminal portion of the cytoplasmic tail caused marked decreases in receptor coupling to phospholipase C. Conservative substitutions of two residues in the C terminus of the third **cytoplasmic loop** (Ala293----Leu, Lys290----His) increased the potency of agonists for stimulating phosphatidylinositol metabolism by up to 2 orders of magnitude. These data indicate (i) that the regions of the alpha 1AR that determine coupling to phosphatidylinositol metabolism are similar to those previously shown to be involved in coupling of beta 2AR to adenylate cyclase stimulation and (ii) that point mutations of a **G-protein**-coupled receptor can cause remarkable increases in sensitivity of biological response.

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=> d his

     (FILE 'HOME' ENTERED AT 17:51:58 ON 30 MAY 2001)

     FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 17:53:36 ON 30 MAY 2001
L1        56521 S G-PROTEIN OR 7TM OR (SEVEN TRANSMEMBRANE) OR (SEVEN
MEMBRANE)
L2         1216 S L1 AND ((CYTOPLASMIC LOOP?) OR (INTRACELLULAR LOOP?))
L3       219027 S L2 AND CHIMER? OR FUSION OR FUSED
L4          232 S L2 AND (CHIMER? OR FUSION OR FUSED)
L5           19 S L4 AND RHODOPSIN
L6            8 DUP REM L5 (11 DUPLICATES REMOVED)
L7          176 S L4 AND PY <1999
L8           79 DUP REM L7 (97 DUPLICATES REMOVED)

=> s bacteriorhodopsin and ((cytoplasmic loop?) or (intracellular loop?))

L9           30 BACTERIORHODOPSIN AND ((CYTOPLASMIC LOOP?) OR (INTRACELLULAR
                LOOP?))

=> dup rem l9

PROCESSING COMPLETED FOR L9
L10          19 DUP REM L9 (11 DUPLICATES REMOVED)

=> d ibib abs l-19
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'L-19' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files. Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> d ibib abs L10 1-19

L10 ANSWER 1 OF 19 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 2001:32417748 BIOTECHNO
TITLE: Cytoplasmic surface structure of **bacteriorhodopsin** consisting of interhelical loops and C-terminal .alpha. helix, modified by a variety of environmental factors as studied by .sup.1.sup.3C-NMR
AUTHOR: Yamaguchi S.; Yonebayashi K.; Konishi H.; Tuzi S.; Naito A.; Lanyi J.K.; Needleman R.; Saito H.
CORPORATE SOURCE: H. Saito, Department of Life Science, Himeji Institute of Technology, Harima Science Garden City, Kouto 3-chome, Kamigori, Hyogo 678-1297, Japan. E-mail: saito@sci.himeji-tech.ac.jp
SOURCE: European Journal of Biochemistry, (2001), 268/8 (2218-2228), 55 reference(s) CODEN: EJBCAI ISSN: 0014-2956
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2001:32417748 BIOTECHNO
AB We have examined the .sup.1.sup.3C-NMR spectra of [3-.sup.1.sup.3C] Ala-labeled **bacteriorhodopsin** and its mutants by varying a variety of environmental or intrinsic factors such as ionic strength, temperature, pH, truncation of the C-terminal .alpha. helix, and site-directed mutation at **cytoplasmic loops**, in order to gain insight into a plausible surface structure arising from the C-terminal .alpha. helix and loops. It is found that the surface structure can be characterized as a complex stabilized by salt bridges or metal-mediated linkages among charged side chains. The surface complex in **bacteriorhodopsin** is most pronounced under the conditions of 10 mm NaCl at neutral pH but is destabilized to yield relaxed states when environmental factors are changed to high ionic strength, low pH and higher temperature. These two states were readily distinguished by associated spectral changes, including suppressed (cross polarization-magic angle spinning NMR) or displaced (upfield) .sup.1.sup.3C signals from the C-terminal .alpha. helix, or modified spectral features in the loop region. It is also noteworthy that such spectral changes, when going from the complexed to relaxed states, occur either when the C-terminal .alpha. helix is deleted or site-directed mutations were introduced at a **cytoplasmic loop**. These observations clearly emphasize that organization of the cytoplasmic surface complex is important in the stabilization of the three-dimensional structure at ambient temperature, and subsequently plays an essential role in biological functions.

L10 ANSWER 2 OF 19 MEDLINE
ACCESSION NUMBER: 2001125504 MEDLINE
DOCUMENT NUMBER: 20580208 PubMed ID: 11141080
TITLE: Solution structure of the second extracellular loop of human thromboxane A2 receptor.
AUTHOR: Ruan K H; So S P; Wu J; Li D; Huang A; Kung J
CORPORATE SOURCE: Vascular Biology Research Center and Division of

atology, Department of Interna edicine, The University of Texas Health Science Center, Houston, Texas 77030, USA..
kruan@imed2010.med.ut.tmc.edu
CONTRACT NUMBER: HL 56712 (NHLBI)
NS 23327 (NINDS)
SOURCE: BIOCHEMISTRY, (2001 Jan 9) 40 (1) 275-80.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered PubMed: 20010125
Entered Medline: 20010222
AB Thromboxane A(2) receptor (TP receptor), a prostanoid receptor, belongs to the G protein-coupled receptor family, composed of three **intracellular loops** and three extracellular loops connecting seven transmembrane helices. The highly conserved extracellular domains of the prostanoid receptors were found in the second extracellular loop (eLP(2)), which was proposed to be involved in ligand recognition. The 3D structure of the eLP(2) would help to further explain the ligand binding mechanism. Analysis of the human TP receptor model generated from molecular modeling based on **bacteriorhodopsin** crystallographic structure indicated that about 12-14 A separates the N- and C-termini of the extra- and **intracellular loops**. Synthetic loop peptides whose termini are constrained to this separation are presumably more likely to mimic the native loop structure than the corresponding loop region peptide with unrestricted ends. To test this new concept, a peptide corresponding to the eLP(2) (residues 173-193) of the TP receptor has been made with the N- and C-termini connected by a homocysteine disulfide bond. Through 2D nuclear magnetic resonance (NMR) experiments, complete (1)H NMR assignments, and structural construction, the overall 3D structure of the peptide was determined. The structure shows two beta-turns at residues 180 and 185. The distance between the N- and C-termini of the peptide shown in the NMR structure is 14.2 A, which matched the distance (14.5 A) between the two transmembrane helices connecting the eLP(2) in the TP receptor model. This suggests that the approach using the constrained loop peptides greatly increases the likelihood of solving the whole 3D structures of the extra- and the intracellular domains of the TP receptor. This approach may also be useful in structural studies of the extramembrane loops of other G protein-coupled receptors.

L10 ANSWER 3 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:244385 BIOSIS
DOCUMENT NUMBER: PREV200100244385
TITLE: Solution structure of the second extracellular loop of human thromboxane A2 receptor.
AUTHOR(S): Ruan, Ke-He (1); So, Shui-Ping (1); Wu, Jiaxin (1); Li, Dawei; Huang, Aimin (1); Kung, Jennifer (1)
CORPORATE SOURCE: (1) University of Texas Health Science Center at Houston, 6431 Fannin, Houston, TX, 77030 USA

SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A202. print.
Meeting Info.: Annual Meeting of the Federation of American
Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

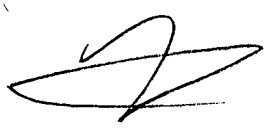
AB Thromboxane A2 receptor (TP receptor), belonged to G protein-coupled receptor family, composes of three **intracellular loops** and three extracellular loops connecting seven transmembrane helices. The highly conserved extracellular domains for the prostanoid receptors were found in second extracellular loop (eLP2), which was proposed to be involved for ligand recognition. 3D structure of the eLP2 would help to further explain the ligand-binding mechanism. Analysis of human TP receptor model based on **bacteriorhodopsin** crystallographic structure indicated 12-14 ANG separation of N- and C-termini of the extra- and **intracellular loops**. Synthetic loop peptides whose termini are constrained to this separation are more likely to mimic the native loop structure than the corresponding loop region peptide with unrestricted ends. To test this new concept, a peptide corresponding to eLP2 (residues 173-193) of TP receptor has been made with the N- and C-termini connected by a homocysteine disulfide bond. Through 2D nuclear magnetic resonance (NMR) experiments, complete 1H NMR assignments and structural construction, the overall 3D structure of the peptide was determined. The structure shows two beta-turns at residue 180 and 185. The distance between the N- and C-termini of the peptide shown in NMR structure is 14.2 ANG, which matched the distance (14.5 ANG) between the two transmembrane helices connecting the eLP2 in the TP receptor model. This suggests that the approach using the constrained loop peptides greatly increases the likelihood of solving the whole 3D structures of the extra- and intracellular domains of TP receptor, and may also be useful in structural studies of the extramembrane loops of other G protein-coupled receptors.

L10 ANSWER 4 OF 19 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001084712 MEDLINE
DOCUMENT NUMBER: 20541405 PubMed ID: 11087400
TITLE: Alteration of conformation and dynamics of **bacteriorhodopsin** induced by protonation of Asp 85 and deprotonation of Schiff base as studied by 13C NMR.
AUTHOR: Kawase Y; Tanio M; Kira A; Yamaguchi S; Tuzi S; Naito A; Kataoka M; Lanyi J K; Needleman R; Saito H
CORPORATE SOURCE: Department of Life Science, Faculty of Science, Himeji Institute of Technology, Harima Science Garden City, Kouto 3-chome, Kamigori, Hyogo, Japan.
SOURCE: BIOCHEMISTRY, (2000 Nov 28) 39 (47) 14472-80.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered PubMed: 20001211
Entered Medline: 20010118

AB According to previous X-ray diffraction studies, the D85N mutant of **bacteriorhodopsin** (bR) with unprotonated Schiff base assumes a protein conformation similar to that in the M photointermediate. We recorded (13)C NMR spectra of [3-(13)C]Ala- and [1-(13)C]Val-labeled D85N and D85N/D96N mutants at ambient temperature to examine how conformation and dynamics of the protein backbone are altered when the Schiff base is

protonated (at 7) and unprotonated (at pH 10) [redacted]ost notably, we found that the peak i nsities of three to four [3-(1[redacted]C]Ala-labeled residues from the transmembrane alpha-helices, including Ala 39, 51, and 53 (helix B) and 215 (helix G), were suppressed in D85N and D85N/D96N both from CP-MAS (cross polarization-magic angle spinning) and DD-MAS (dipolar decoupled-magic angle spinning) spectra, irrespective of the pH. This is due to conformational change and subsequent acquisition of intermediate time-range motions, with correlation times in the order of 10(-)(5) or 10(-)(4) s, which interferes with proton decoupling frequency or frequency of magic angle spinning, respectively, essential for an attempted peak-narrowing to achieve high-resolution NMR signals. Greater changes were achieved, however, at pH 10, which indicate large-amplitude motions of transmembrane helices upon deprotonation of Schiff base and the formation of the M-like state in the absence of illumination. The spectra detected more rapid motions in the extracellular and/or **cytoplasmic loops**, with correlation times increasing from 10(-)(4) to 10(-)(5) s. Conformational changes in the transmembrane helices were located at helices B, G, and D as viewed from the above-mentioned spectral changes, as well as at 1-(13)C-labeled Val 49 (helix B), 69 (B-C loop), and [3-(13)C]Ala-labeled Ala 126 (D-helix) signals, in addition to the cytoplasmic and extracellular loops. Further, we found that in the M-like state the charged state of Asp 96 at the cytoplasmic side substantially modulated the conformation and dynamics of the extracellular region through long-distance interaction.

L10 ANSWER 5 OF 19 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000411048 MEDLINE
DOCUMENT NUMBER: 20335025 PubMed ID: 10873864
TITLE: Conformations of the rhodopsin third **cytoplasmic loop** grafted onto **bacteriorhodopsin.**
AUTHOR: Heymann J B; Pfeiffer M; Hildebrandt V; Kaback H R; Fotiadis D; Groot B; Engel A; Oesterhelt D; Muller D J
CORPORATE SOURCE: M.E. Muller-Institute for Structural Biology, Biozentrum, University of Basel, Basel, CH-4056, Switzerland.
SOURCE: STRUCTURE WITH FOLDING & DESIGN, (2000 Jun 15) 8 (6) 643-53.
Journal code: DEB; 100889329. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000907
Last Updated on STN: 20000907
Entered Medline: 20000830



AB BACKGROUND: The third **cytoplasmic loop** of rhodopsin (Rho EF) is important in signal transduction from the retinal in rhodopsin to its G protein, transducin. This loop also interacts with rhodopsin kinase, which phosphorylates light-activated rhodopsin, and arrestin, which displaces transducin from light-activated phosphorylated rhodopsin. RESULTS: We replaced eight residues of the EF loop of **bacteriorhodopsin** (BR) with 24 residues from the third **cytoplasmic loop** of bovine Rho EF. The surfaces of purple membrane containing the mutant BR (called IIIN) were imaged by atomic force microscopy (AFM) under physiological conditions to a resolution of 0.5-0.7 nm. The crystallinity and extracellular surface of IIIN were not perturbed, and the cytoplasmic surface of IIIN increased in height compared with BR, consistent with the larger loop. Ten residues of Rho EF were excised by V8 protease, revealing helices E and F in the AFM topographs. Rho EF was modeled onto the BR structure, and the envelope derived from the AFM data of IIIN was used to select probable models. CONCLUSIONS: A likely conformation of Rho EF involves some extension of helices E and F, with the tip of the loop lying over helix C and projecting towards the C terminus. This is consistent with mutagenesis data showing the TTQ transducin-binding motif close to loop CD, and cysteine cross-linking data indicating the C-terminal part of Rho EF to be

close to the CD loop.

L10 ANSWER 6 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:465981 BIOSIS
DOCUMENT NUMBER: PREV200000465981
TITLE: Unraveling conformational changes of **bacteriorhodopsin** using pairs of spin labels.
AUTHOR(S): Radzwill, Nicole (1); Gerwert, Klaus (1); Steinhoff, Heinz-Juergen (1)
CORPORATE SOURCE: (1) Max-Planck-Institute of Molecular Physiology, Otto-Hahn-Strasse 11, 44227, Dortmund Germany
SOURCE: European Biophysics Journal, (2000) Vol. 29, No. 4-5, pp. 318. print.
Meeting Info.: 3rd European Biophysics Congress Munchen, Germany September 09-13, 2000
ISSN: 0175-7571.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L10 ANSWER 7 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:101834 BIOSIS
DOCUMENT NUMBER: PREV200100101834
TITLE: Solution structure of the second extracellular loop of human thromboxane A2 receptor.
AUTHOR(S): Ruan, Ke-He (1); So, Shui-Ping; Wu, Jiaxin; Li, Dawei; Huang, Aimin; Kung, Jennifer
CORPORATE SOURCE: (1) Division of Hematology, Department of Internal Medicine, University of Texas Health Science Center at Houston, 6431 Fannin St., Houston, TX, 77030: kruan@imed2010.med.uth.tmc.edu USA
SOURCE: Biochemistry, (January 9, 2000) Vol. 40, No. 1, pp. 275-280. print.
ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Thromboxane A2 receptor (TP receptor), a prostanoid receptor, belongs to the G protein-coupled receptor family, composed of three **intracellular loops** and three extracellular loops connecting seven transmembrane helices. The highly conserved extracellular domains of the prostanoid receptors were found in the second extracellular loop (eLP2), which was proposed to be involved in ligand recognition. The 3D structure of the eLP2 would help to further explain the ligand binding mechanism. Analysis of the human TP receptor model generated from molecular modeling based on **bacteriorhodopsin** crystallographic structure indicated that about 12-14 ANG separates the N- and C-termini of the extra- and **intracellular loops**. Synthetic loop peptides whose termini are constrained to this separation are presumably more likely to mimic the native loop structure than the corresponding loop region peptide with unrestricted ends. To test this new concept, a peptide corresponding to the eLP2 (residues 173-193) of the TP receptor has been made with the N- and C-termini connected by a homocysteine disulfide bond. Through 2D nuclear magnetic resonance (NMR) experiments, complete 1H NMR assignments, and structural construction, the overall 3D structure of the peptide was determined. The structure shows two beta-turns at residues 180 and 185. The distance between the N- and C-termini of the peptide shown in the NMR structure is 14.2 ANG, which matched the distance (14.5 ANG) between the two transmembrane helices connecting the eLP2 in the TP receptor model. This suggests that the approach using the constrained loop

peptides greatly increases the likelihood of solving the whole 3D structures of the extra- and the intracellular domains of the TP receptor.

This approach may also be useful in structural studies of the extramembrane loops of other G protein-coupled receptors.

L10 ANSWER 8 OF 19 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000237157 MEDLINE
DOCUMENT NUMBER: 20237157 PubMed ID: 10773169
TITLE: High-field EPR studies of the structure and conformational changes of site-directed spin labeled **bacteriorhodopsin**.
AUTHOR: Steinhoff H; Savitsky A; Wegener C; Pfeiffer M; Plato M; Mobius K
CORPORATE SOURCE: Lehrstuhl fur Biophysik, Ruhr-Universitat Bochum, 44780, Bochum, Germany.. hjs@bph.ruhr-uni-biochum.de
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (2000 Apr 21) 1457 (3) 253-62.
Journal code: A0W; 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000608

AB Cw and pulsed high-field EPR (95 GHz, 3.4 T) are performed on site-directed spin labeled **bacteriorhodopsin** (BR) mutants. The enhanced Zeeman splitting leads to spectra with resolved g-tensor components of the nitroxide spin label. The g(xx) component shift determined for 10 spin labels located in the **cytoplasmic loop** region and in the protein interior along the BR proton channel reveals a maximum close to position 46 between the proton donor D96 and the retinal. A plot of g(xx) versus A(zz) of the nitrogen discloses grouping of 12 spin labeled sites in protic and aprotic sites. Spin labels at positions 46, 167 and 171 show the aprotic character of the cytoplasmic moiety of the proton channel whereas nitroxides at positions 53, 194 and 129 reveal the protic environment in the extracellular channel. The enhanced sensitivity of high-field EPR with respect to anisotropic reorientational motion of nitroxides allows the characterization of different motional modes for spin labels bound to positions 167 and 170. The motional restriction of the nitroxide at position 167 of the double mutant V167C/D96N is decreased in the M(N) photo-intermediate. An outward shift of the cytoplasmic moiety of helix F in the M(N) intermediate would account for the high-field EPR results and is in agreement with diffraction and recent X-band EPR data.

L10 ANSWER 9 OF 19 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001162868 MEDLINE
DOCUMENT NUMBER: 21161775 PubMed ID: 11263244
TITLE: Molecular modeling on human CCR5 receptors and complex with CD4 antigens and HIV-1 envelope glycoprotein gp120.
AUTHOR: Yang J; Liu C Q
CORPORATE SOURCE: Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China.
SOURCE: Acta Pharmacol Sin, (2000 Jan) 21 (1) 29-34.
Journal code: DPS; 100956087.
PUB. COUNTRY: China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered PubMed: 20010323

Entered Medline: 20010503

AB AIM: To investigate the interaction between human CCR5 receptors (CCR5) and HIV-1 envelope glycoprotein gp120 (HIV-1 gp120) and HIV-1 receptor CD4 antigens (CD4). METHODS: The structurally conserved regions (SCR) of human CCR5 was built by the SYBYL/Biopolymer module using the corresponding transmembrane (TM) domain of **bacteriorhodopsin** (bR) as the template. The coordinates for amino-terminal residue sequence, and carboxyl-terminal residue sequence, extracellular and **cytoplasmic loops** were generated using LOOP SEARCH algorithm. Subsequently the structural model was merged into the complex with HIV-1 gp120 and CD4. RESULTS: Human CCR5 interacted with both an HIV-1 gp120 and CD4. The N-terminal residues (especially Met1 and Gln4) of human CCR5 contacted with CD4 residues, mainly with one span (56-59) of CD4 in electrostatic interaction and hydrogen-bonds. The binding sites of human CCR5 were buried in a hydrophobic center surrounded by a highly basic periphery. On the other hand, direct interatomic contacts were made between 7 CCR5 residues and 6 gp120 amino-acid residues, which included van der Waals contacts, hydrophobic interaction, and hydrogen bonds. CONCLUSION: The interaction model should be helpful for rational design of novel anti-HIV drugs.

L10 ANSWER 10 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:135267 BIOSIS
DOCUMENT NUMBER: PREV200000135267
TITLE: AFM imaging and modelling of the rhodopsin third **cytoplasmic loop** grafted onto **bacteriorhodopsin**.
AUTHOR(S): Heymann, Bernard (1); Pfeiffer, Matthias; Hildebrandt, Volker; Fotiadis, Dimitrios (1); de Groot, Bert; Engel, Andreas (1); Buldt, Georg; Oesterhelt, Dieter; Muller, Daniel J.
CORPORATE SOURCE: (1) University of Basel, Klingelbergstrasse 70, Basel, CH-4056 Switzerland
SOURCE: Biophysical Journal., (Jan., 2000) Vol. 78, No. 1 Part 2, pp. 6A.
Meeting Info.: 44th Annual Meeting of the Biophysical Society. New Orleans, Louisiana, USA February 12-16, 2000
ISSN: 0006-3495.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L10 ANSWER 11 OF 19 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999396617 MEDLINE
DOCUMENT NUMBER: 99396617 PubMed ID: 10465768
TITLE: Conformational changes of **bacteriorhodopsin** along the proton-conduction chain as studied with (13)C NMR of [3-(13)C]Ala-labeled protein: arg(82) may function as an information mediator.
AUTHOR: Tanio M; Tuzi S; Yamaguchi S; Kawaminami R; Naito A; Needleman R; Lanyi J K; Saito H
CORPORATE SOURCE: Department of Life Science, Himeji Institute of Technology, Harima Science Garden City, Kuoto 3-chome, Kamigori, Hyogo, 678-1297, Japan.
SOURCE: BIOPHYSICAL JOURNAL, (1999 Sep) 77 (3) 1577-84.
Journal code: A5S; 0370626. ISSN: 0006-3495.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991008

AB We have recorded (13)C NMR spectra of [3-(13)C]Ala-labeled wild-type

bacteriorhodops(bR) and its mutants at Arg(82) Asp(85), Glu(194), and G(204) along the extracellular p on transfer chain. The upfield and downfield displacements of the single carbon signals of Ala(196) (in the F-G loop) and Ala(126) (at the extracellular end of helix D), respectively, revealed conformational differences in E194D, E194Q, and E204Q from the wild type. The same kind of conformational change at Ala(126) was noted also in the Y83F mutant, which lacks the van der Waals contact between Tyr(83) and Ala(126) present in the wild type. The absence of a negative charge at Asp(85) in the site-directed mutant D85N induced global conformational changes, as manifested in displacements or suppression of peaks from the transmembrane helices, **cytoplasmic loops**, etc., as well as the local changes at Ala(126) and Ala(196) seen in the other mutants. Unexpectedly, no conformational change at Ala(126) was observed in R82Q (even though Asp(85) is protonated at pH 6) or in D85N/R82Q. The changes induced in the Ala(126) signal when Asp(85) is uncharged could be interpreted therefore in terms of displacement of the positive charge of Arg(82) toward Tyr(83), where Ala(126) is located. It is possible that disruption of the proton transfer chain after protonation of Asp(85) in the photocycle could cause the same kind of conformational change we detect at Ala(196) and Ala(126). If so, the latter change would be also the result of rearrangement of the side chain of Arg(82).

L10 ANSWER 12 OF 19 MEDLINE
ACCESSION NUMBER: 1999341233 MEDLINE
DOCUMENT NUMBER: 99341233 PubMed ID: 10412722
TITLE: **Bacteriorhodopsin** in a periodic boundary water-vacuum-water box as an example towards stable molecular dynamics simulations of G-protein coupled receptors.
AUTHOR: ter Laak A M; Kuhne R
CORPORATE SOURCE: Forschungsinstitut fur Molekulare Pharmakologie, Berlin, Germany.. terlaak@fmp-berlin.de
SOURCE: RECEPTORS AND CHANNELS, (1999) 6 (4) 295-308. Journal code: B3Y; 9315376. ISSN: 1060-6823.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19991005
Last Updated on STN: 20000303
Entered Medline: 19990923

AB This study presents an optimised set-up for molecular dynamics (MD) simulations of G-protein coupled receptors (GPCR). Such simulations are complicated because (1) the experimental template structure for GPCRs (bovine rhodopsin) is of low resolution, (2) the receptor surroundings are irregular (water exposed loops vs. lipid exposed transmembrane regions) and (3) the protonation and solvation states of the inner core receptor residues are unknown. We compared various simulations of the experimentally derived and refined electron density structure of the seven helical transmembrane protein **bacteriorhodopsin** (bR) under different MD conditions using AMBER 4.1. Our results demonstrate that the optimal MD set-up with minimal computational effort is a periodic boundary (PB) box containing two water shells solvating the extra- and **intracellular loops** separated by a vacuum layer surrounding the helical transmembrane (TM) regions. It was found that the vacuum layer and water layers are stable under periodic boundary conditions during at least 1 ns of MD simulation. In this set-up the bR structure is stable without any restraints. The average bR structure during the last 500 ps of the MD run has an excellent RMSD value relative to the original bR structure (RMSD = 1.66 A for the C alpha atoms within the TM domains) and shows a very high helical stability within the TM

regions (88.8% [redacted]ix). The use of this MD set-up [redacted]r simulations of GPCRs is discussed.

L10 ANSWER 13 OF 19 MEDLINE
ACCESSION NUMBER: 1998118960 MEDLINE
DOCUMENT NUMBER: 98118960 PubMed ID: 9457616
TITLE: The cannabinoid receptor: computer-aided molecular modeling and docking of ligand.
AUTHOR: Mahmoudian M
CORPORATE SOURCE: Department of Pharmacology, Iran University of Medical Sciences, Tehran, Iran.. massoud@nrcgeb.ac.ir
SOURCE: JOURNAL OF MOLECULAR GRAPHICS AND MODELLING, (1997 Jun) 15 (3) 149-53, 179.
Journal code: CVM; 9716237. ISSN: 1093-3263.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980319
Last Updated on STN: 19980319
Entered Medline: 19980311

AB A three-dimensional model of human cannabinoid receptor is constructed using computer-aided molecular modeling techniques. The helices of **bacteriorhodopsin** were used as the initial template to construct the transmembrane helices. The extracellular and **intracellular loops** were added using the SYBYL molecular modeling package. The extracellular N terminus was modeled on the basis of its similarity to rat oncomodulin. Similarly, the C terminus was constructed on the basis of similarity to bovine prothrombin fragment 1. The final structure was refined by several runs of minimization and dynamics calculation using the CHARMm package. delta 9-Tetra hydrocannabinol was docked into the internal cavity using the AUTODOCK program. Our study snows that there may be a calcium-binding site in the extracellular N terminus of this receptor. The ligand binds mainly to a hydrophobic site, which consists of residues Met-240, Trp-241 (TMH-4), Trp-356, Leu-359, Leu-360 (TMH-6), and Ala-283 (TMH-5). Its phenolic hydroxyl group forms a hydrogen bond with the carboxy group of Ala-198 (TMH-3). The results of modeling agree well with experimental QSAR studies.

L10 ANSWER 14 OF 19 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 95302480 MEDLINE
DOCUMENT NUMBER: 95302480 PubMed ID: 7783190
TITLE: Force-induced conformational change of **bacteriorhodopsin**.
AUTHOR: Muller D J; Buldt G; Engel A
CORPORATE SOURCE: M. E. Muller-Institute for Structural Biology Biozentrum, University of Basel, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1995 Jun 2) 249 (2) 239-43.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950726
Last Updated on STN: 19970203
Entered Medline: 19950718

AB The cytoplasmic surface topography of purple membranes imaged by the atomic force microscope depends mainly on the force applied to the stylus.
Imaged at forces of 300 pN, individual **bacteriorhodopsin** molecules reveal two domains. The resulting donut-shaped trimers reversibly transform into structures exhibiting three prominent

protrusions when scanned at 100 pN. In parallel, height of the protein moiety above the lipid layer increases from 4 A to 6 A. From the known structure of **bacteriorhodopsin** it appears that this change may be related to a bending of the most prominent **cytoplasmic loop**.

L10 ANSWER 15 OF 19 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 95034803 MEDLINE
DOCUMENT NUMBER: 95034803 PubMed ID: 7947777
TITLE: Covalently bound pH-indicator dyes at selected extracellular or cytoplasmic sites in **bacteriorhodopsin**. 1. Proton migration along the surface of **bacteriorhodopsin** micelles and its delayed transfer from surface to bulk.
AUTHOR: Scherrer P; Alexiev U; Marti T; Khorana H G; Heyn M P
CORPORATE SOURCE: Department of Physics, Freie Universitat Berlin, Germany.
CONTRACT NUMBER: GM 28289 (NIGMS)
SOURCE: BIOCHEMISTRY, (1994 Nov 22) 33 (46) 13684-92.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 20000303
Entered Medline: 19941221

AB The kinetics of the light-induced release and uptake of protons was monitored with the optical pH-indicator fluorescein covalently bound to various sites on the extracellular and cytoplasmic surfaces of **bacteriorhodopsin**. Selective labeling was achieved by reacting (iodoacetamido)fluorescein with the single cysteine residues in **bacteriorhodopsin** introduced at the desired positions by site-directed mutagenesis. All measurements were performed with **bacteriorhodopsin** micelles in phospholipid/detergent mixtures in 150 mM KCl at 22 degrees C, pH 7.3. Neither the replacements by cysteine nor the subsequent labeling affected the absorption spectrum of **bacteriorhodopsin** and the rise times of the M intermediate. Only the decay of M was altered for some **bacteriorhodopsin** mutants with cysteine residues on the cytoplasmic side. The proton release time detected with fluorescein attached to the extracellular surface (the proton release side) at position 72 (in the loop connecting helices B and C) or 130 (DE loop) was 22 +/- 4 microseconds, clearly faster than that measured with pyranine in the aqueous bulk phase (125 +/- 10 microseconds for wild-type and all mutants studied). For **bacteriorhodopsin** mutants labeled at positions 35, 101, 160, 229, and 231 in the **cytoplasmic loop** region (the proton uptake side), the released proton was observed with a time of 61 +/- 4 microseconds. This was about 3-fold slower than the release time on the extracellular side, but still significantly faster than that measured with pyranine in the bulk phase. These results suggest that the released protons are retained on the micellar surface and move more rapidly along this surface to the cytoplasmic side than from the surface to the bulk medium.(ABSTRACT TRUNCATED AT 250 WORDS)

L10 ANSWER 16 OF 19 MEDLINE
ACCESSION NUMBER: 95046281 MEDLINE
DOCUMENT NUMBER: 95046281 PubMed ID: 7957880
TITLE: A cytoplasmic domain is required for the functional interaction of SRI and HtrI in archaeal signal transduction.
AUTHOR: Krah M; Marwan W; Oesterhelt D
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: FEBS LETTERS, (1994 Oct 24) 353 (3) 301-4.
Journal code: EUH; 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: [redacted]ority Journals
ENTRY MONTH: [redacted]411
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 19970203
Entered Medline: 19941129

AB Phototaxis in the archaeon Halobacterium salinarium is mediated by a stable complex of the photoreceptor sensory rhodopsin I and its transducer HtrI, which relays the light stimulus to the signalling pathway. Removal of the cytoplasmic signalling domain of HtrI eliminated the SRI-specific motor response to light stimulation and led to the loss of the spectroscopically detectable physical interaction of SRI and HtrI. A similar phenotype was obtained by deleting part of a **cytoplasmic loop** located between the second transmembrane helix of HtrI and the signalling domain. These results indicate that the photochemical behavior of sensory rhodopsin I is not determined by interaction with the transmembrane helices of HtrI per se but functionally coupled to the signalling domain. It is proposed that light excitation of SRI results in a conformational change of the transducer which is conducted by the **cytoplasmic loop**, an extra module not found in the eubacterial transducer homologues, and activates the signalling domain.

L10 ANSWER 17 OF 19 MEDLINE
ACCESSION NUMBER: 94281156 MEDLINE
DOCUMENT NUMBER: 94281156 PubMed ID: 8011597
TITLE: The complex of human Gs protein with the beta 3 adrenergic receptor: a computer-aided molecular modeling study.
AUTHOR: Mahmoudian M
CORPORATE SOURCE: Department of Pharmacology, University of Medical Science, Tehran, Iran.
SOURCE: JOURNAL OF MOLECULAR GRAPHICS, (1994 Mar) 12 (1) 22-8, 34. Journal code: AZB; 9014762. ISSN: 0263-7855.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940810
Last Updated on STN: 20000303
Entered Medline: 19940728

AB Three-dimensional (3D) models of the human Gs protein, the human beta 3 adrenergic receptor and their complex are constructed using computer-aided molecular modeling techniques. The structures of bacterial EF-tu 200 and **bacteriorhodopsin** were used as starting points for modeling the Gs protein and beta 3 receptor, respectively. Experimental data are used as constraints to guide the modeling. The resulting 3D structures of the Gs protein, the beta 3 receptor and their complex are in accord with the experimental data. It is found that the third **intracellular loop** of the beta 3 receptor as well as its C-terminus are involved in the binding. Various residues of N-terminus and C-terminus of the Gs protein also participate in the binding. The model of the complex suggests that the Gs protein binds to the beta 3 receptor in such a way that it will be placed in the interface of membrane and intracellular space. This orientation is supported by experimental data. It is concluded that the modeled structure of the complex of the alpha subunit of the human Gs protein and the beta 3 adrenergic receptor is in agreement with the experimental data and it can provide a basis for understanding the way these proteins interact.

L10 ANSWER 18 OF 19 MEDLINE
ACCESSION NUMBER: 93180578 MEDLINE
DOCUMENT NUMBER: 93180578 PubMed ID: 7680090
TITLE: Structure/function relationships of muscarinic acetylcholine receptors.
AUTHOR: Brann M R; Klimkowski V J; Ellis J
CORPORATE SOURCE: Department of Psychiatry, University of Vermont, Burlington

0205.
CONTRACT NUMBER: [redacted] AG05214 (NIA)
SOURCE: LIFE SCIENCES, (1993) 52 (5-6) 405-12. Ref: 39
Journal code: L62; 0375521. ISSN: 0024-3205.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930416
Last Updated on STN: 19960129
Entered Medline: 19930401

AB The regions of muscarinic receptors that specify G-protein-coupling and ligand-binding have been defined in several recent studies. Overall, these studies have shown that amino acids within the third **cytoplasmic loop** of the receptors define their selectivity for different G-proteins, and that multiple, discontinuous epitopes contribute to their selectivities for different ligands. In fact, several competitive and allosteric antagonists can be classified into groups based on which of these epitopes contribute to their subtype selectivity. Site-directed mutagenesis, combined with covalent-labeling studies have suggested that ligands bind to a hydrophobic core of the receptors that is formed by multiple transmembrane (TM) domains. An aspartic acid located in TM3 is likely to bind to the ammonium headgroup of muscarinic ligands, and multiple hydroxyl-containing amino acids contribute to agonist but not antagonist binding. These data are discussed in the context of a computational model of a muscarinic receptor. Our model is based on a sequence alignment with **bacteriorhodopsin**, a seven TM protein for which a higher resolution structure is available. Most of the mutagenic data can be rationalized in the context of this model, and predict testable hypotheses concerning the mechanism by which ligands control the activity of muscarinic receptors.

L10 ANSWER 19 OF 19 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 90148925 MEDLINE
DOCUMENT NUMBER: 90148925 PubMed ID: 2575917
TITLE: Substitution of membrane-embedded aspartic acids in **bacteriorhodopsin** causes specific changes in different steps of the photochemical cycle.
AUTHOR: Stern L J; Ahl P L; Marti T; Mogi T; Dunach M; Berkowitz S;
Rothschild K J; Khorana H G
CORPORATE SOURCE: Department of Chemistry, Massachusetts Institute of Technology, Cambridge 02139.
CONTRACT NUMBER: AI11479 (NIAID)
GM28289-09 (NIGMS)
SOURCE: BIOCHEMISTRY, (1989 Dec 26) 28 (26) 10035-42.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19970203
Entered Medline: 19900327

AB Millisecond photocycle kinetics were measured at room temperature for 13 site-specific **bacteriorhodopsin** mutants in which single aspartic acid residues were replaced by asparagine, glutamic acid, or alanine. Replacement of aspartic acid residues expected to be within the membrane-embedded region of the protein (Asp-85, -96, -115, or -212) produced large alterations in the photocycle. Substitution of Asp-85 or Asp-212 by Asn altered or blocked formation of the M410 photointermediate.
Substitution of these two residues by Glu decreased the amount of M410 formed. Substitutions of Asp-96 slowed the decay rate of the M410

photointermediate and substitutions of Asp-115 slowed the decay rate of the O640 photointermediate. Corresponding substitutions of aspartic acid residues expected to be in **cytoplasmic loop** regions of the protein (Asp-36, -38, -102, or -104) resulted in little or no alteration of the photocycle. Our results indicate that the defects in proton pumping which we have previously observed upon substitution of Asp-85, Asp-96, Asp-115, and Asp-212 [Mogi, T., Stern, L. J., Marti, T., Chao, B. H., & Khorana, H. G. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 4148-4152] are closely coupled to alterations in the photocycle. The photocycle alterations observed in these mutants are discussed in relation to the functional roles of specific aspartic acid residues at different stages of the **bacteriorhodopsin** photocycle and the proton pumping mechanism.

=> log y